Analysis of Antiestrogen Drugs by HPLC

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Abstract

Antiestrogen drugs are used to inhibit the estardiol aromatasis enzyme which is responsible for the biosynthesis of estrogen from testosterone. The growth of certain tumors, for example, some types of breast cancer, is related to the concentration of estrogen in the adjacent tissue. By inhibiting the enzyme the concentration of estrogen can be reduced thus decreasing the growth of the tumor cells. The inhibition can be done competitively (competitive inhibitor) or completely (suicide inhibitor). Most aromatasis inhibitors have a testosterone-like structure where one methyl group is replaced. Tamoxifen is a non-steroid type inhibitor.

Figure 1 shows the chromatogram of tamoxifen using gradient analysis on a reversed phase column and UV detection. To avoid decomposition of samples the autosampler temperature was set to 4 ºC.

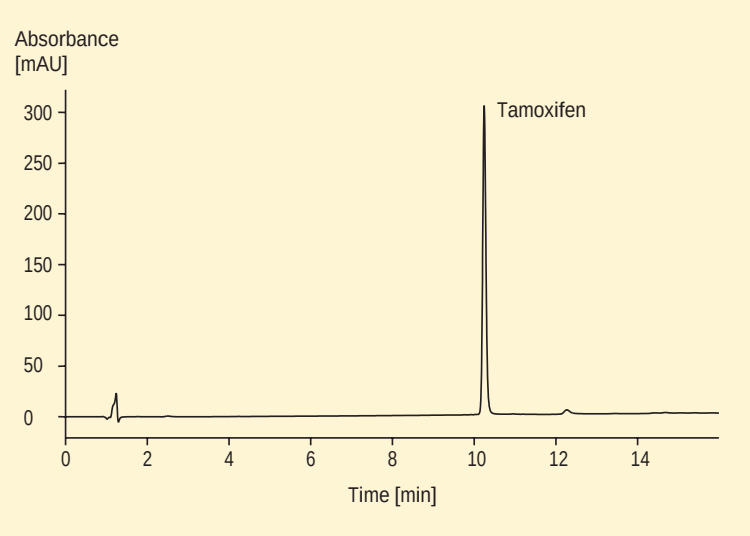

Figure 1
Analysis of tamoxifen

Conditions

Column
4 x 125 mm Purospher RP-18 5 µm
Mobile phase
A = 0.025 M KH_2PO_4 in water
(pH = 3 with H_2SO_4)
B = acetonitrile
Flow rate 1.0 ml/min
Gradient 20 % B to 60 % B in 14 min
Column wash
60 % B to 20 % B in 2 min
UV detector
variable wavelength detector 204 nm, standard cell
Column compartment temperature
60 °C
Stop time 16 min
Post time 5 min
Injection volume 5 µl

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The performance of the HPLC method is shown in the table below.

Compound	LOD for S/N=2 (mg/l)*	Precision of RT (RSD of 10 runs) (100 mg/l)*	Precision of Area (RSD of 10 runs) (100 mg/l)*
Tamoxifen	0.2	0.08	0.46

* Injection volume: 5 µl

The HPLC method presented here shows an easy but reliable and precise analysis of the antiestrogen drug tamoxifen. The values for LOD, precision of RT and precision of area show the good performance of the analysis

Equipment

Agilent 1100 Series

- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell, 10-mm path length, 13-µl cell volume

Alternative:

- Vacuum degasser
- Binary pump
- Diode-array detector, standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

Columns

- Purospher RP-18, 5 µm, 4 x 125 mm (Agilent part number 79925PU-564)
- *Recommended:* Guard cartridges Purosphere RP-18, 5 µm, 4 x 12.5 mm (Agilent part number 79925PU-504, 10/pk)

Note:
Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. To avoid sample decomposition it is necessary to use a cooled autosampler, for example, the Agilent 1100 Series thermostatted autosampler.

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Released 08/98
Publication Number 5968-1118E

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